**Extended experimental procedures**

Cell Counting and statistical analysis

Analysis was performed on cryosectioned coronal tissues at 20 µm for embryonic ages and 25 µm for postnatal ages. To determine cells per area, the Image-J cell counter plugin was used to count the number of cells from either a 4x or 10x objective image that included all layers of the neocortex and this number was divided by the area of the neocortex from the image to calculate cells/mm$^2$. For striatum, hippocampal and globus pallidus counts, a 4x objective was use to capture each structure and then counted in the same manner. The % of tdTomato$^+$ cells that co-labeled with a particular marker was calculated by dividing the number of co-labeled cells by the total number of tdTomato$^+$ cells to determine values. For cell transplants, all tdTomato$^+$ cells were counted in the neocortex from all sections in the rostral to caudal series. For cell fate counts, only transplants where at least 50 tdTomato$^+$ cells could be counted were used for analysis. For soma size quantification, tdTomato$^+$ cell soma's in the neocortex were traced in Image J. For each transplant, at least 25 cells were measured and averaged. Cells from all neocortical layers were measured from multiple images. Statistics were performed using Prism version 6, a *p* value of $< 0.05$ was considered significant.

DNA vector generation

*DlxI12b-βG-Cre-T2a vectors*

The *DlxI12b-βG* fragment was PCR amplified with introduced 5' XhoI and 3' NheI sites (5' GAGACTCGAGACACAGCTTAATGATTATC, 3' GAGAGCTAGCCGCCGCGCTCTGCTTCTG) and ligated into a *CMV-GFP-T2a-MCS* lentiviral backbone, replacing the *CMV* promoter. Next *Cre* was PCR amplified with 5' NheI and 3' BsrGI sites (5'

GAGAGCTAGCATGGCCAATTTACTGACC, 3' GAGATGTACAGCACCGGTCCATCGCCATC) and ligated in frame to the T2a element after excising GFP with NheI and BsrGI. Next, human *Pten* cDNA was acquired from Origene, and used as a template to introduce ASD mutations. Extension overlap PCR was used to introduce *Pten* mutations using the following primers with introduced mutations (underlined) in combination with flanking primers (below): (H93>R: 5' CAATATCCTTTTGAAGACCGTAACCCACCACAGCTAG, 3' CTAGCTGTGGTGGGTTACGGTCTTCAAAAGGATATTG; H118>P: CTAAGTGAAGATGACAATCCTGTTGCAGCAATTCAC, 3' GTGAATTGCTGCAACAGGATTGTCATCTTCACTTAG; Y176>C: 5' GAGGCGCTATGTGTATTGTTATAGCTACCTGTTAAAG, 3' CTTTAACAGGTAGCTATAACAATACACATAGCGCCTC; F241>S: 5' GAAGACAAGTTCATGTACTCTGAGTTCCCTCAGCCG, 3' CGGCTGAGGGAACTCAGAGTACATGAACTTGTCTTC; D252>G: 5' CGTTACCTGTGTGTGGTGGTATCAAAGTAGAGTTC, 3' GAACTCTACTTTGATACCACCACACACAGGTAACG), underlined nucleotides indicate mutated residue. First, two PCR products were generated with the primers: (5' flanking and 3' mutant, 5' mutant and 3' flanking), using human Pten cDNA as a template. Next, these PCR products were combined together and PCR amplified using flanking primers with introduced SphI sites (5' GAGAGCATGCATGACAGCCATCATCAAAG, 3' GAGAGCATGCTCAGACTTTTGTAATTTG) These full length products were then ligated 3' and in frame to the T2a element into the SphI site of the *DlxI12b-βG-Cre-T2a-MCS* lentiviral vector. All vectors were then verified by restriction digest and sequence verified.

EdU labeling

Pregnant mice were pulsed with EdU (10mg/ml) at a dose of 50mg Edu/kg body weight. After 30 minutes, mice were sacrificed and embryos collected in ice-cold PBS (pH 7.2). Embryos were

put in 4% PFA and fixed overnight at 4°C, and sunk in 30% sucrose before embedding in OCT. $Edu^+$ cells were visualized by following standard procedures in the Clik-iT EdU plus kit (life technologies) and then co-stained with DAPI.

Electrophysiology

250 µm coronal slices from 3-6 week-old mice of either sex were prepared. ACSF contained (in mM): 126 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 $NaH_2PO_4$, 1 $MgCl_2$, 2 CaCl, and 10 glucose.

*Intracellular recordings of spontaneous inhibitory currents*

We obtained somatic whole-cell patch recordings using a Multiclamp 700A (Molecular Devices) and differential contrast video microscopy on an upright microscope (BX51WI; Olympus). Patch electrodes (tip resistance = 2–6 MOhms) were filled with the following (in mM): $CsCH_4O_3S$ (130), $MgCl_2$ (2), $NaCl_2$ (4), ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (10), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (10), adenosine 5′-triphosphate (2), guanosine 5′-triphosphate (0.5), tetraethylammonium (5), N-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium (5), QX-314-Cl (5) and 0.3% (w/v) biocytin. All recordings were at 32.5±1°C. Series resistance was usually 10–20 MΩ, and experiments were discontinued above 30 MΩ. Layer II/III pyramidal neurons were first identified by their pyramidal shape in DIC and then screened for tdTomato fluorescence to avoid patching *Nkx2.1cre*-lineage interneurons. Spontaneous inhibitory currents were recorded with the neurons voltage-clamped at +10mV. Mini Analysis (Synaptosoft) was used to detect inhibitory currents; analysis was performed blind to genotype.

Image acquisition and analysis:

Fluorescent images were taken using a Coolsnap camera (Photometrics) mounted on a Nikon Eclipse 80i microscope using NIS Elements acquisition software (Nikon). Images of mice and brains were taken with an iphone5 (Apple). Brightness and contrast were adjusted and images merged using Image-J software.

Immunofluorescent tissue staining:

Immunofluorescent labeling was performed on 25 µm (P0 and older) or 20 µm (embryonic ages) cryosections with the following primary antibodies: rabbit anti-cleaved-caspase-3 (Cell signaling), rabbit anti-Kv3.1b (Chemicon), rabbit anti-NPAS1 (gift from Steven McKnight, University of Texas Southwestern Medical Center), rabbit anti-PH3 (Millipore), rabbit anti-parvalbumin (Swant, PV25), rabbit anti-PTEN (Cell Signaling), goat anti-somatostatin (SantaCruz), rat anti-somatostatin (Millipore), rabbit anti-VIP (Immunostar). 488, 594 or 647 Alexa-conjugated secondary antibodies (Life Technologies) were used. Sections were coverslipped with Vectashield containing DAPI (Vector labs).

Intracellular recordings of transplanted MGE-derived interneurons:

We obtained somatic whole-cell patch recordings using a Multiclamp 700A (Molecular Devices) and differential contrast video microscopy on an upright microscope (BX51WI; Olympus). For current clamp experiments, patch electrodes (tip resistance = 2–6 MOhms) were filled with the following (in mM): 130 K-gluconate, 10 KCl, 10 HEPES, 10 EGTA, 2 MgCl, 2 MgATP, and 0.3 NaGTP (pH adjusted to 7.3 with KOH). All recordings were at 32.5±1°C. Series resistance was usually 10–20 MΩ, and experiments were discontinued above 30 MΩ.

*Electrophysiologic classification of transplanted MGE-derived interneurons*

For experiments of cell-autonomous effects of *Pten* deletion using the MGE transplant assay, E12.5 transplanted MGE cells from *DlxI12b-Cre; Pten$^{Flox}$; Ai14$^{Flox}$* embryos were identified via tdTomato expression. Since fast-spiking parvalbumin$^{+}$ interneurons have minimal spike adaptation compared to somatostatin$^{+}$ interneurons (Kawaguchi and Kubota, 1996; Kawaguchi 1993), cells were separated into accommodating and non-accommodating cells based on their adaptation ratio (i.e. ratio between the first and last interspike interval was <1.5).

Lentivirus preparation

HEK293T cells grown in DMEM H21 with 10% FBS were transfected using Lipofectamine$^{2000}$ (Invitrogen) with four plasmids to generate lentivirus particles as previously described (Vogt et al., 2014; Vogt et al., 2015). Plasmids used for a 10 cm tissue culture dish of HEK293T cells at about 50-70% confluency: 6.4 µg of Lentiviral vector DNA, with 1.2 µg each of 3 helper plasmids (*pVSV-g*, *pRSVr* and *pMDLg-pRRE*). Media was completely replaced 4 hours after transfection, and cells were grown for four days before harvesting. On day four of culture, all the media was collected and filtered through a 0.45 low protein binding membrane to remove cells and large debris. The filtered media was pooled and ultracentrifuged at 100,000 x g for 2.5 hours at 4°C. After the ultracentrifuge step, supernatant was removed and the pellet was resuspended overnight at 4°C in sterile PBS then stored at -80°C until use.

MGE cell transplantation

Lentiviral labeling of MGE cells before transplantation was performed as previously described (Vogt et al., 2014; Vogt et al., 2015). E12.5 MGEs from individual embryos were dissected in

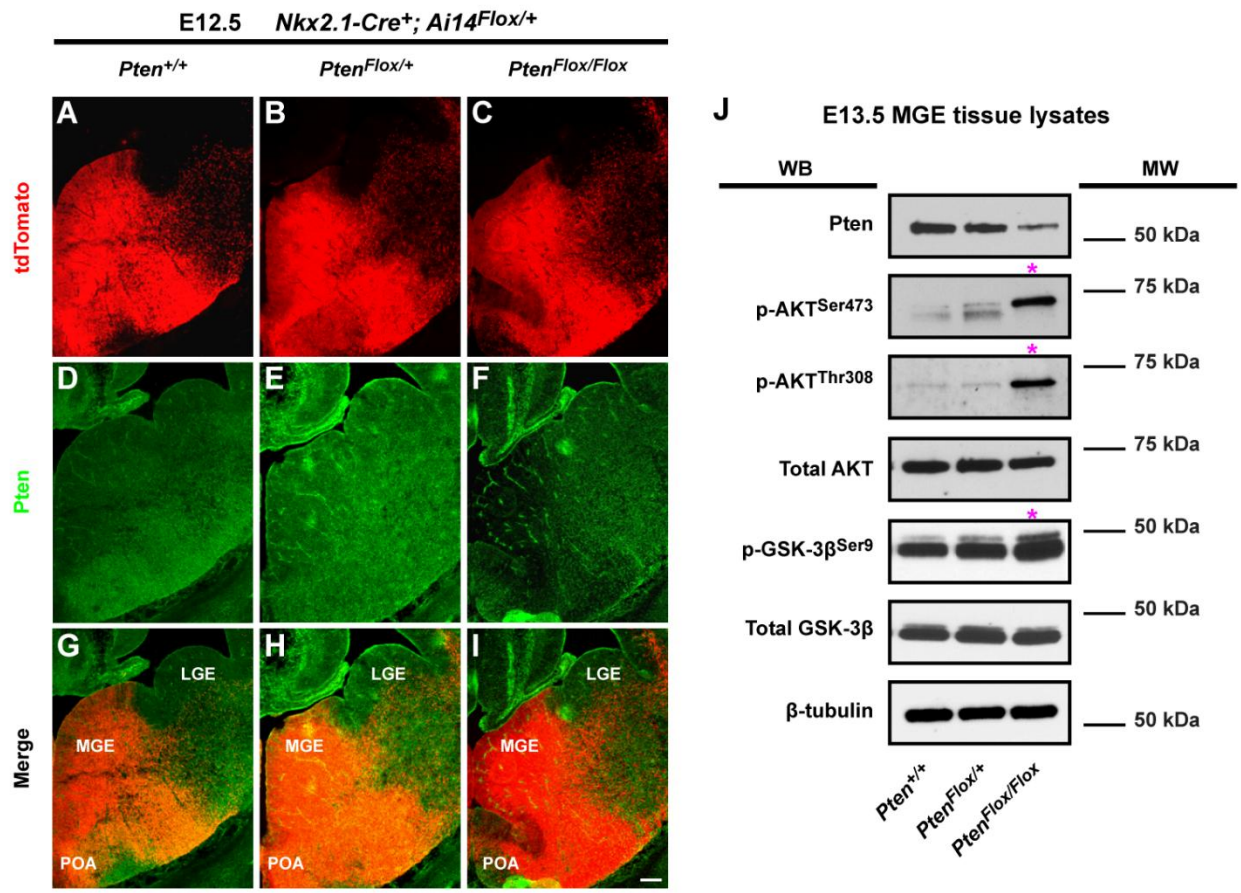
ice-cold HBSS and then kept on ice in DMEM media (containing 10% fetal bovine serum). MGEs were then mechanically dissociated with a p1000 pipette tip and then either concentrated for injections or infected with lentiviruses. For lentiviral infections, dissociated MGE cells were mixed with pre-warmed media, polybrene (8 µg/ml), and about 10-20 µls of concentrated lentiviruses, and incubated at 37°C for 30 minutes, with intervals of agitation. Since the MGE cells had a Cre-dependent reporter, Ai14 (Madisen et al., 2010), only MGE cells transduced with Cre-expressing lentiviruses would be visible in the host neocortex after transplantation. This combinatorial method (Vogt et al., 2015) allows the transduced/transplanted cells to express a strong reporter, i.e. tdTomato expressed from the beta-actin promoter, while maintaining cell type specificity. Cells were then pelletted in a tabletop centrifuge at low speed (700xg, 3-4 minutes) and washed 3 times with media followed by trituration to disperse cells between each wash to remove excess virus. The final cell pellet was covered by 2-3 µl of media, put on ice, and then remaining media was removed before the injection needle was loaded using a sterile paper towel. For injections, a glass micropipette of 50 µm diameter (with a beveled tip) was preloaded with sterile mineral oil and cells were front-loaded into the tip of the needle using a plunger connected to a hydraulic drive (Narishige) that was mounted to a stereotaxic frame. Pups were anesthetized on ice for 1-2 minutes before being placed on the mold for injections. Each pup received 2-4 injections of cells (70 nl per site), in the right hemisphere. These sites were about 1 mm apart from rostral to caudal and were injected into layers V-VI of a P1 neocortex. After injections, pups were allowed recover and then put back with the mother. Mice were sacrificed at 35 days after transplant and transcardially perfused with PBS followed by 4% PFA. Brains were then postfixed in 4% PFA and sunk in 30% sucrose before embedding in OCT.

Western analysis

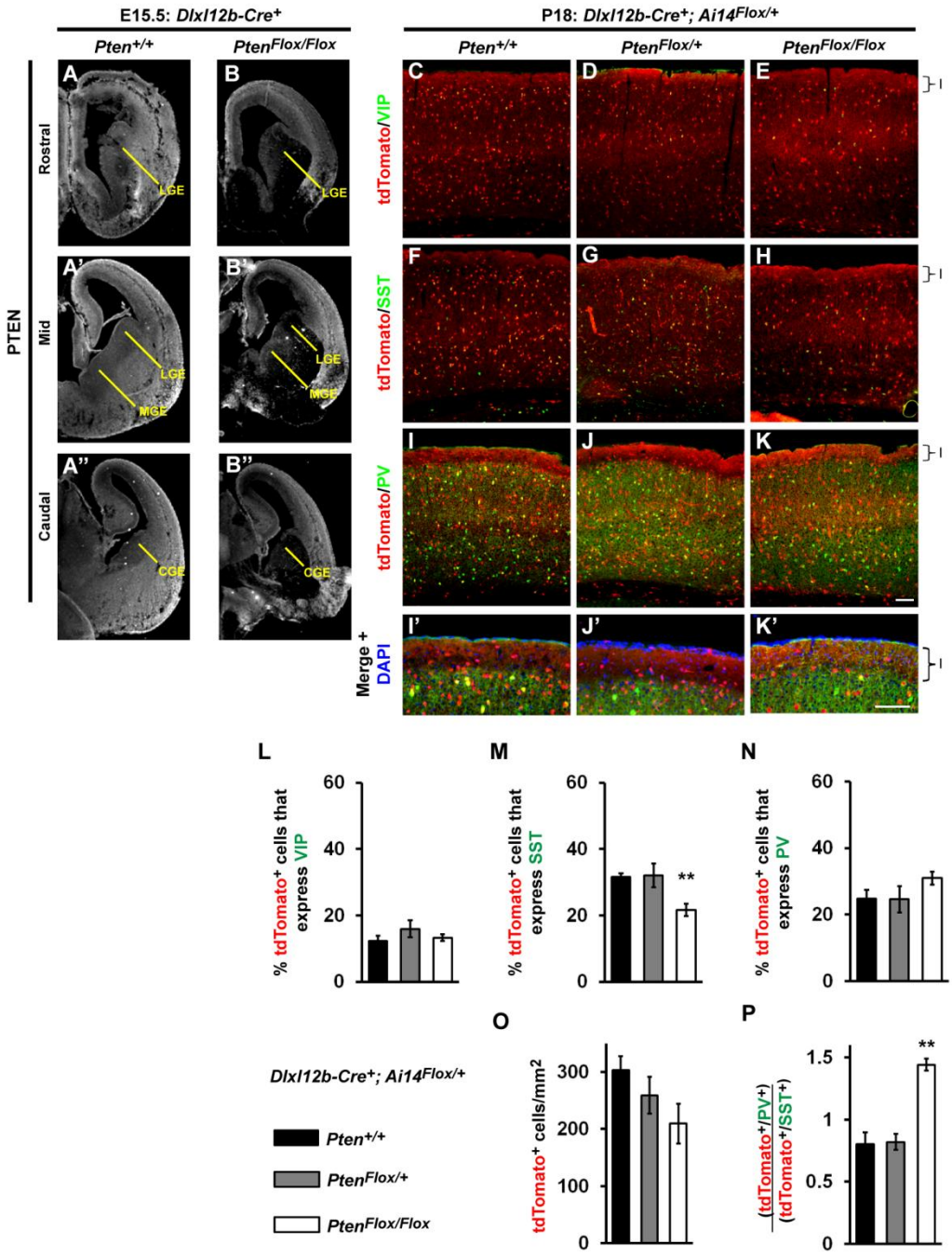
HEK293T and MGE tissue were harvested in RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% sodium deoxycholate in 50 mM Tris) with protease (Halt protease inhibitor, Pierce) and phosphatase (PhosSTOP, Roche) inhibitors, then ~ 20 µg of total protein was separated in SDS-PAGE gels and transferred to nitrocellulose membranes. Western blotting was performed by standard procedures. Antibodies used: rabbit anti-AKT (Cell Signaling), rabbit anti-pAKT$^{Thr308}$ (Cell Signaling), mouse anti-pAKT$^{Ser473}$ (Cell Signaling), rabbit anti-GSK3beta (SantaCruz), rabbit anti-pGSK3beta$^{Ser9}$ (Biosource), mouse anti-betaIII-tubulin (Covance), rabbit anti-Cre (Novagen) and rabbit anti-PTEN (Cell Signaling). Rabbit and mouse HRP-conjugated secondary antibodies were from Biorad.

## Supplemental Figure and Tables

### Figure S1



## Figure S2


E15.5: Dlxl12b-Cre+
Pten+/+
PtenFlox/Flox
P18: Dlxl12b-Cre+; Ai14Flox/+
Pten+/+
PtenFlox/+
PtenFlox/Flox
PTEN
Rostral
Mid
Caudal
LGE
MGE
CGE
tdTomato/VIP
tdTomato/SST
tdTomato/PV
Merge + DAPI
% tdTomato+ cells that express VIP
% tdTomato+ cells that express SST
% tdTomato+ cells that express PV
tdTomato+ cells/mm2
(tdTomato+/PV+) / (tdTomato+/SST+)
Dlxl12b-Cre+; Ai14Flox/+
Pten+/+
PtenFlox/+
PtenFlox/Flox

## Figure S3

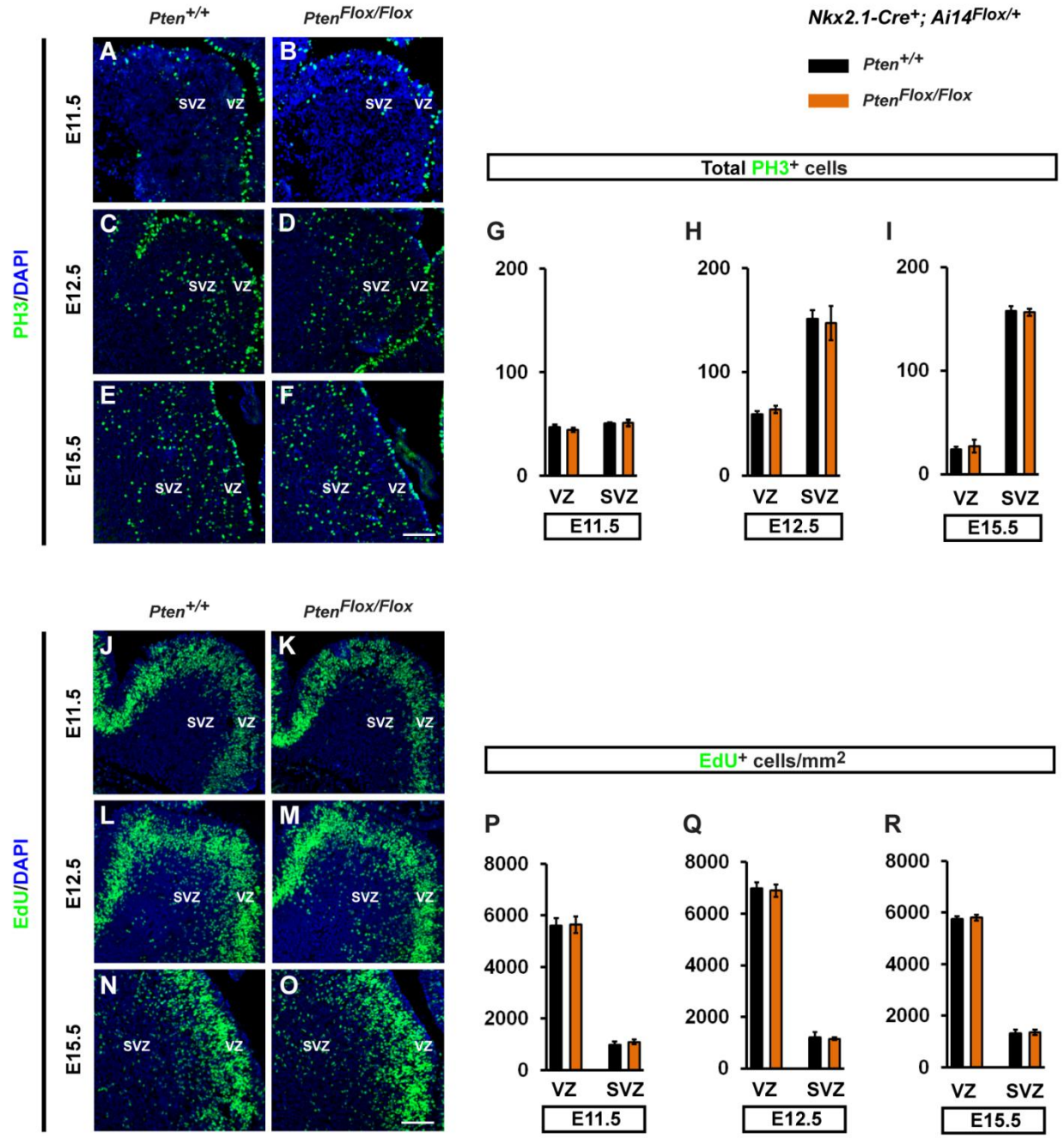

Pten+/+
PtenFlox/Flox
Nkx2.1-Cre+; Ai14Flox/+
Pten+/+
PtenFlox/Flox
PH3/DAPI
E11.5
E12.5
E15.5
A
B
C
D
E
F
SVZ
VZ
Total PH3+ cells
G
H
I
200
100
0
VZ
SVZ
E11.5
E12.5
E15.5
EdU/DAPI
J
K
L
M
N
O
EdU+ cells/mm2
P
Q
R
8000
6000
4000
2000
0

**Figure S4**

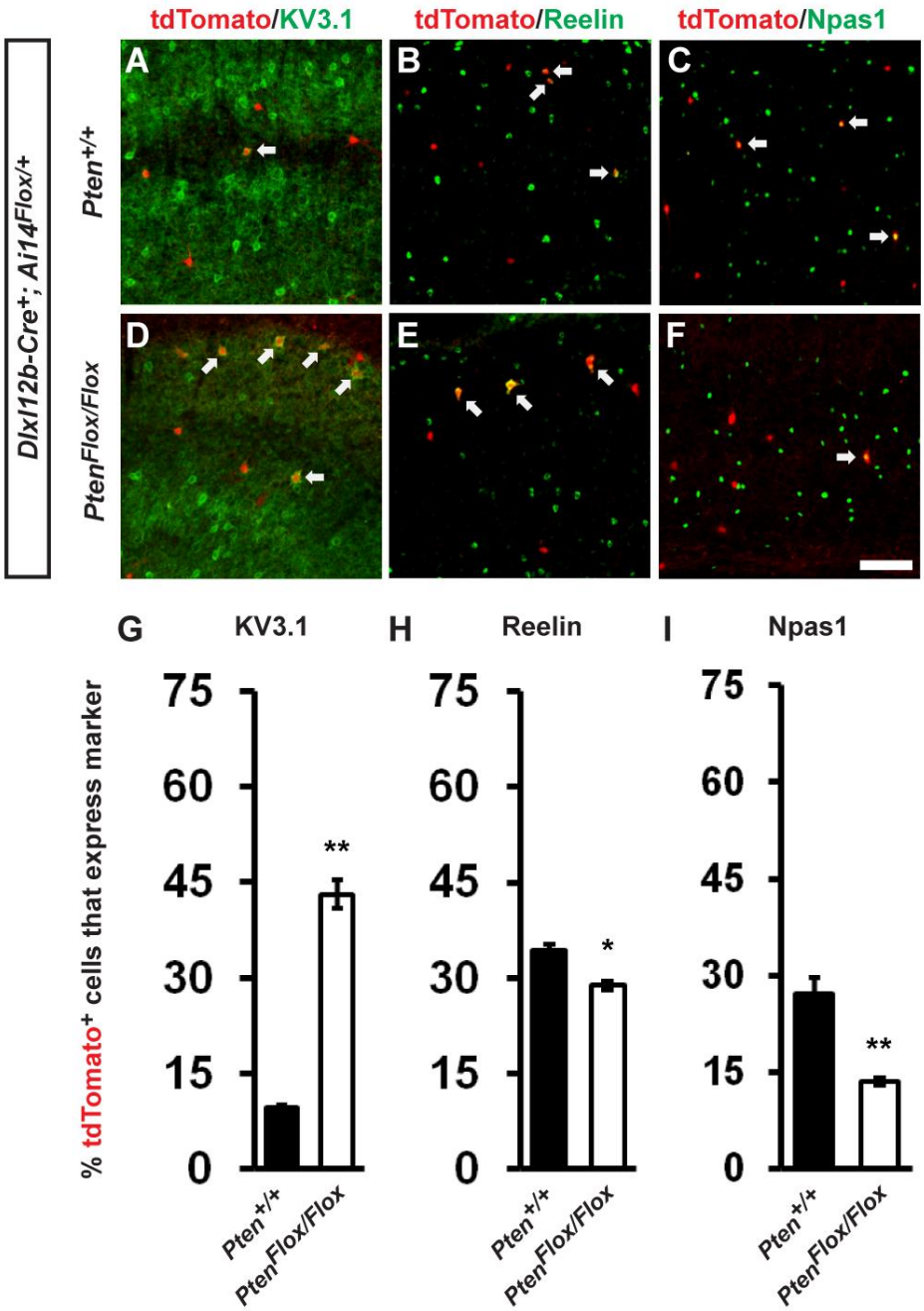
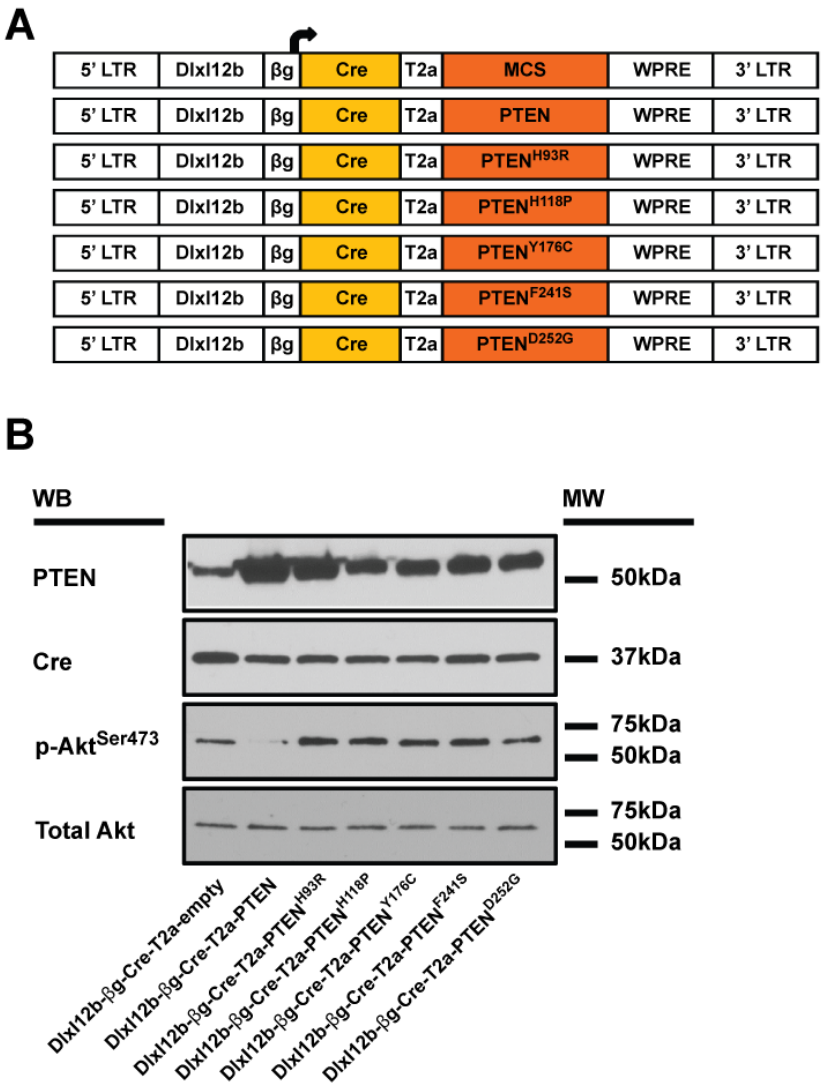

tdTomato/KV3.1
tdTomato/Reelin
tdTomato/Npas1
Dlx12b-Cre+; Ai14Flox/+
Pten+/+
PtenFlox/Flox
A
B
C
D
E
F
G
KV3.1
H
Reelin
I
Npas1
% tdTomato+ cells that express marker
75
60
45
30
15
0
**
*
**
Pten+/+
PtenFlox/Flox

**Figure S5**


A
5' LTR
Dlxl12b
βg
Cre
T2a
MCS
PTEN
PTEN^H93R
PTEN^H118P
PTEN^Y176C
PTEN^F241S
PTEN^D252G
WPRE
3' LTR
B
WB
MW
PTEN
Cre
p-Akt^Ser473
Total Akt
50kDa
37kDa
75kDa
50kDa
75kDa
50kDa
Dlxl12b-βg-Cre-T2a-empty
Dlxl12b-βg-Cre-T2a-PTEN
Dlxl12b-βg-Cre-T2a-PTEN^H93R
Dlxl12b-βg-Cre-T2a-PTEN^H118P
Dlxl12b-βg-Cre-T2a-PTEN^Y176C
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## Figure S6

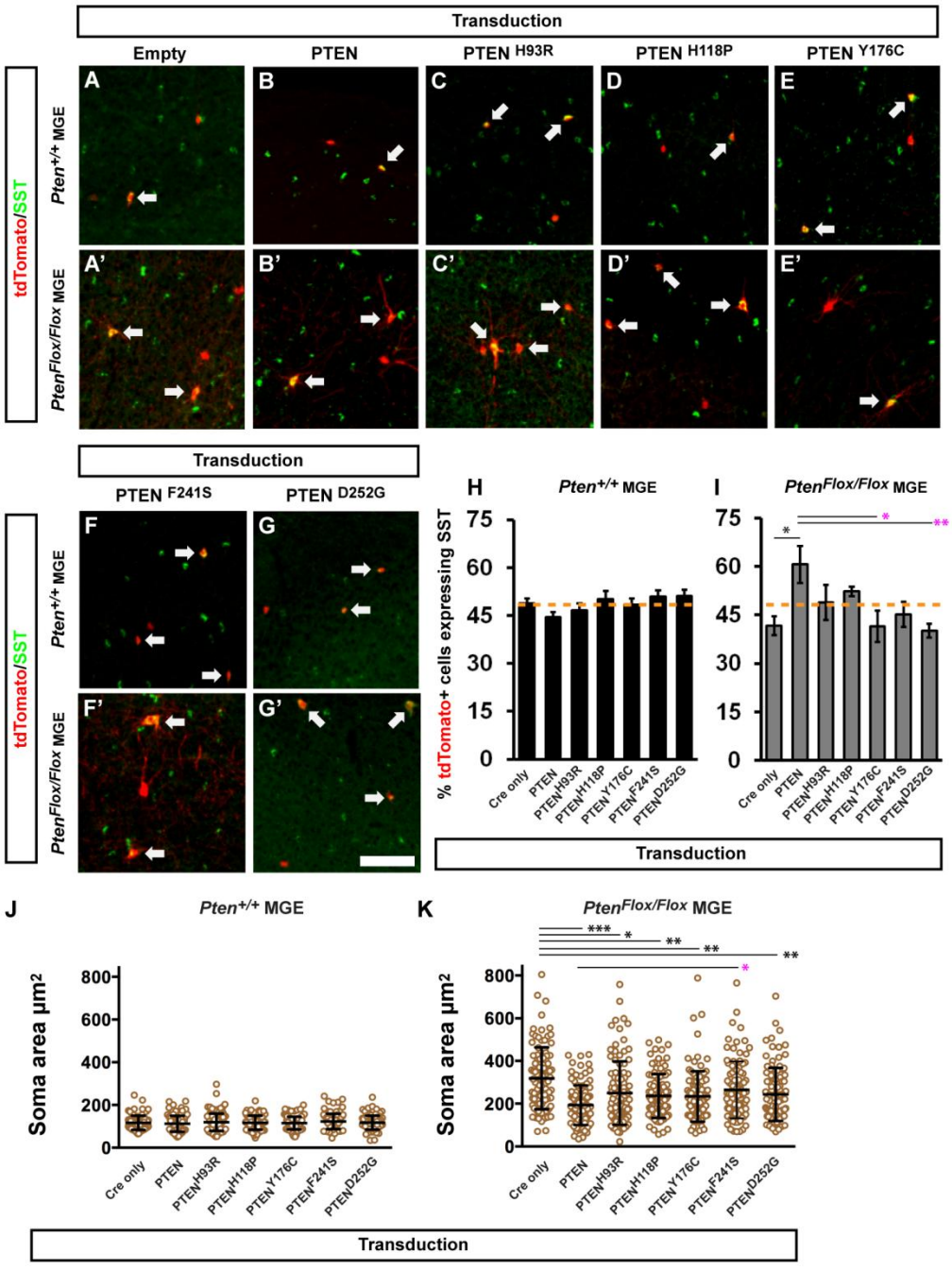
Transduction
Empty
PTEN
PTEN H93R
PTEN H118P
PTEN Y176C
tdTomato/SST
Pten+/+ MGE
PtenFlox/Flox MGE
A
B
C
D
E
A'
B'
C'
D'
E'
Transduction
PTEN F241S
PTEN D252G
F
G
F'
G'
H
Pten+/+ MGE
I
PtenFlox/Flox MGE
% tdTomato+ cells expressing SST
75
60
45
30
15
0
Cre only
PTEN
PTENH93R
PTENH118P
PTEN Y176C
PTENF241S
PTEND252G
Transduction
J
Pten+/+ MGE
K
PtenFlox/Flox MGE
Soma area µm²
800
600
400
200
0
Transduction

**Table S1**

| | WT | *Pten* cKO | p value |
|---|---|---|---|
| **Tissue** | **Total *Nkx2.1-Cre*-lineage cells/mm$^2$** | | |
| Hippocampus | 632.3 ± 46.4 | 400.0 ± 24.0 | ** 0.0043 |
| Neocortex | 213 ± 4.7 | 102.2 ± 7.8 | **** < 0.0001 |
| Striatum | 860.8 ± 34.0 | 315.8 ± 27.0 | **** < 0.0001 |
| Globus pallidus | 2054.5 ± 57.2 | 1881.1 ± 150.4 | n.s. 0.2791 |

| **Marker** | **Tissue** | **Total *Nkx2.1-Cre*-lineage$^+$/marker$^+$ cells/mm$^2$** | | |
|---|---|---|---|---|
| **PV** | Hippocampus | 192.1 ± 16.0 | 157.6 ± 34.8 | n.s. 0.4025 |
| | Neocortex | 65.6 ± 5.7 | 41.4 ± 3.3 | * 0.0107 |
| | Striatum | 202.8 ± 27.7 | 132.3 ± 18.6 | n.s. 0.0656 |
| | Globus pallidus | 990.6 ± 55.7 | 989.1 ± 100.6 | n.s. 0.9883 |
| **SST** | Hippocampus | 181.9 ± 16.1 | 57.4 ± 8.6 | *** 0.0005 |
| | Neocortex | 94.7 ± 9.8 | 40.9 ± 3.0 | ** 0.0021 |
| | Striatum | 127.6 ± 9.2 | 31.5 ± 12.8 | ** 0.0037 |
| **ChAT** | Striatum | 138.7 ± 11.7 | 61.9 ± 10.2 | ** 0.0078 |
| | Globus pallidus | 143.6 ± 10.7 | 119.0 ± 10.9 | n.s. 0.1842 |

| **Marker** | **Tissue** | **% *Nkx2.1-Cre*-lineage cells that express marker** | | |
|---|---|---|---|---|
| **PV** | Hippocampus | 25.3 ± 2.7 | 37.1 ± 4.1 | n.s. 0.0586 |
| | Neocortex | 33.5 ± 0.9 | 46.7 ± 3.4 | * 0.0101 |
| | Striatum | 24.0 ± 4.0 | 38.4 ± 3.0 | * 0.027 |
| | Globus pallidus | 48.2 ± 1.9 | 52.5 ± 2.3 | n.s. 0.2059 |
| **SST** | Hippocampus | 28.0 ± 1.1 | 14.5 ± 2.3 | ** 0.0017 |
| | Neocortex | 58.6 ± 5.8 | 38.8 ± 2.1 | * 0.0189 |
| | Striatum | 14.8 ± 1.0 | 8.4 ± 1.7 | * 0.0316 |
| **ChAT** | Striatum | 19.5 ± 1.7 | 16.2 ± 1.8 | n.s. 0.256 |
| | Globus pallidus | 7.2 ± 0.2 | 6.0 ± 1.5 | n.s. 0.4488 |

| **Tissue** | **Ratio of PV/SST** | | |
|---|---|---|---|
| Hippocampus | 1.0 ± 0.2 | 2.7 ± 0.2 | *** 0.0009 |
| Neocortex | 0.8 ± 0.1 | 1.3 ± 0.01 | * 0.0151 |
| Striatum | 1.5 ± 0.1 | 4.0 ± 0.6 | * 0.0148 |

**Table S2**

| | | Neocortex | Neocortex | E12.5 MGE transplant | E12.5 MGE transplant |
|---|---|---|---|---|---|
| | | ***Nkx2.1-Cre*** | ***Dlxl12b-Cre*** | ***Dlxl12b-Cre*** | ***Dlxl12b-Cre* lentivirus** |
| **% tdTomato that are PV+** | **WT** | 33.5 ± 0.9 | 24.9 ± 2.6 | 19.3 ± 2.9 | 12.7 ± 0.5 |
| | **cKO** | 46.7 ± 3.4 | 30.9 ± 1.9 | 37.7 ± 5.1 | 26.0 ± 1.6 |
| | **% Change** | ▲ 39.4 | ▲ 24.1 | ▲ 95.3 | ▲ 104.7 |
| **% tdTomato that are SST+** | **WT** | 58.6 ± 5.8 | 32.0 ± 1.9 | 55.9 ± 2.3 | 49.0 ± 1.5 |
| | **cKO** | 38.8 ± 2.1 | 23.8 ± 2.5 | 35.0 ± 2.6 | 41.6 ± 2.9 |
| | **% Change** | ▼ 33.8 | ▼ 25.7 | ▼ 37.4 | ▼ 15.2 |
| **PV/SST ratio** | **WT** | 0.6 ± 0.06 | 0.8 ± 0.1 | 0.35 ± 0.05 | 0.26 ± 0.01 |
| | **cKO** | 1.2 ± 0.06 | 1.44 ± 0.05 | 1.06 ± 0.08 | 0.63 ± 0.05 |

▲ Increased after *Pten* deletion

▼ Decreased after *Pten* deletion

## Table S3

| | Non-accommodating neurons | Mean | SEM | P value | Accommodating neurons | Mean | SEM | P value |
|---|---|---|---|---|---|---|---|---|
| AP threshold (mV) | WT (n=11) | -41.03 | 1.53 | 0.45 | WT (n=11) | -34.04 | 1.75 | *0.001 |
| | cKO (n=9) | -43.45 | 2.53 | | cKO (n=18) | -40.86 | 0.98 | |
| Inter-spike-interval (ms) | WT | 6.39 | 1.01 | 0.87 | WT | 9.86 | 1.37 | 0.11 |
| | cKO | 6.62 | 0.97 | | cKO | 13.15 | 1.32 | |
| FI-slope (Hz/current) | WT | 7.07 | 0.56 | 0.54 | WT | 2.11 | 0.71 | 0.58 |
| | cKO | 6.25 | 1.31 | | cKO | 2.48 | 0.26 | |
| Adaption ratio (last ISI/first ISI) | WT | 1.04 | 0.04 | 0.88 | WT | 3.70 | 1.16 | 0.91 |
| | cKO | 1.06 | 0.12 | | cKO | 3.60 | 0.46 | |
| Resting membrane (mV) | WT | -69.28 | 1.28 | **0.009 | WT | -68.64 | 1.01 | 0.80 |
| | cKO | -74.41 | 1.17 | | cKO | -68.99 | 0.87 | |
| Mean AP half-width (ms) | WT | 0.40 | 0.03 | 0.86 | WT | 0.77 | 0.11 | 0.38 |
| | cKO | 0.41 | 0.04 | | cKO | 0.67 | 0.05 | |
| Membrane resistance (mOhm) | WT | 79.02 | 7.62 | 0.72 | WT | 148.77 | 13.16 | 0.91 |
| | cKO | 84.87 | 15.20 | | cKO | 146.43 | 15.26 | |

**Supplemental Figure and Table Legends**

**Figure S1: Deletion of *Pten* from GABAergic interneuron progenitors in the MGE results in hyper-activation of AKT. Related to Figure 1.**

(A-I) Immunofluorescent images of E12.5 coronal telencephalic hemisections show that Pten protein expression (green) is eliminated from the MGE and POA in the *Nkx2.1-Cre* expression domains, (defined by tdTomato expression). $Pten^{+/+}$ (A, D and G), $Pten^{Flox/+}$ (B, E and H) and $Pten^{Flox/Flox}$ (C, F and I). (J) Western analysis of E13.5 MGE tissues show increased phosphorylation of AKT and GSK3b in $Pten^{Flox/Flox}$; $*p < 0.05$. Abbreviations: LGE and MGE (lateral and medial ganglionic eminences), POA (preoptic area), WB (western blot), MW (molecular weight). Scale bar in (I) = 100 µm.

**Figure S2: *DlxI12b-Cre*$^{+}$; $Pten^{Flox/Flox}$ cKOs have an increased PV/SST ratio and ectopic PV$^{+}$ projections into neocortical layer I. Related to Figures 2 and 3.**

Coronal Immunofluorescent images show PTEN expression in *DlxI12b-Cre*$^{+}$ E15.5 sections of $Pten^{+/+}$ (A-A'') and $Pten^{Flox/Flox}$ (B-B''). Images show a loss of PTEN protein in the MGE, LGE and CGE. Coronal immunofluorescent images of P18 somatosencory cortex for *DlxI12b-Cre* lineage cells (tdTomato$^{+}$) that coexpress VIP (C-E), SST (F-H) or PV (I-K). (I'-K') Higher magnification images of neocortical layer I from PV images I-K, merged with DAPI. (Brackets to the right of images indicate the boundaries of layer I). Quantification of the % tdTomato$^{+}$ cells that co-express VIP (L), SST (M) or PV (N). (O) Quantification of the total number of tdTomato$^{+}$ cells per mm$^{2}$ in the somatosensory cortex. (P) Ratio of tdTomato$^{+}$/PV$^{+}$ cells per mm$^{2}$ over

tdTomato$^{+}$/SST$^{+}$ cells per mm$^{2}$ in the neocortex. Data are represented as mean ± SEM, ***p* < 0.01. Scale bars in (K) and (K') = 100 µm.

**Figure S3: *Nkx2.1-Cre*; *Pten* mutants exhibit normal MGE proliferation at embryonic ages. Related to Figure 1.**

Coronal immunofluorescent images of E11.5 (A, B), E12.5 (C, D), and E15.5 (E, F) MGE regions stained for phospho-histone3 (PH3) from *Pten*$^{+/+}$ and *Pten*$^{Flox/Flox}$ sections. (G-I) Quantification of the total number of PH3$^{+}$ cells in the VZ and SVZ of the MGE at each age. Coronal immunofluorescent images of E11.5 (J, K), E12.5 (L, M), and E15.5 (N, O) MGE regions that were pulsed with EdU. (P-R) Quantification of the number of EdU$^{+}$ cells per mm$^{2}$ in the VZ and SVZ of the MGE at each age. Data are represented as mean ± SEM. Scale bars in (F) and (O) = 100 µm. Abbreviations: (VZ) ventricular zone, (SVZ) subventricular zone, (MZ) marginal zone.

**Figure S4: Transplanted *DlxI12b-Cre*$^{+}$; *Pten*$^{Flox/Flox}$ MGE cells exhibit disproportionate ratios of KV3.1, Reelin and NPAS1. Related to Figure 6.**

E12.5 *DlxI12b-Cre*$^{+}$*; AI14*$^{Flox/+}$ MGE cells from either *Pten*$^{+/+}$ or *Pten*$^{Flox/Flox}$ embryos were transplanted into P1 WT neocortices and assessed at 35 days post transplant (DPT). Coronal immunofluorescent images show tdTomato co-expressed with KV3.1 (A and D), Reelin (B and E) or NPAS1 (C and F) in the neocortex. Quantification for the % of tdTomato$^{+}$ cells that coexpress KV3.1 (G), Reelin (H) or NPAS1 (I). Data are represented as mean ± SEM, **p* < 0.05, ***p* < 0.01. Scale bar in (F) = 100 µm.

**Figure S5: Lentiviral vectors to assess the function of *PTEN* ASD alleles. Related to Figure 7.**

(A) Schema depicting lentiviral constructs to conditionally delete *Pten* and simultaneously express candidate human alleles. (B) Western analysis from HEK293T cell lysates that were transfected with lentiviral DNA vectors. Lysates were harvested two days post transfection and were cultured in the presence of 10% fetal bovine serum. Abbreviations: (βg) beta globin minimal promoter, (LTR) long terminal repeat, (WPRE) woodchuck hepatitis post-transcriptional regulatory element, (WB) western blot, (MW) molecular weight.

**Figure S6: Complementation assay at 35 DPT of *PTEN* ASD alleles to complement the ratio of $SST^+$ cells and soma size. Related to Figure 7.**

E12.5 control (*Pten*$^{+/+}$*; AI14*$^{Flox/+}$) or mutant (*Pten*$^{Flox/Flox}$*; AI14*$^{Flox/+}$) MGE cells were transduced with a *DlxI12b-Cre-T2a-MCS* lentivirus that expressed either *Cre* only, or *Cre* with a human WT *PTEN*, or a *PTEN* ASD allele. MGE cells were transduced with lentiviruses and than transplanted into WT P1 host neocortices and assessed at 35 days post transplant (DPT). Coronal immunofluorescent images showed transplanted and transduced MGE cells (tdTomato$^+$) that coexpress SST for control (A-G) or *Pten*$^{Flox/Flox}$ (A'-G') at 35 DPT. Quantification of the % of tdTomato$^+$ control (H) or *Pten*$^{Flox/Flox}$ (I) transduced-MGE cells that co-express SST. Soma areas were measured using tdTomato immunoflourescence from control (J) and *Pten*$^{Flox/Flox}$ (K) MGE cells that were transduced with *DlxI12b-Cre* lentiviruses. Data are represented as mean ± SEM (H, I) or SD (J, K), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Black asterisks indicate comparisons to *Cre* only, magenta asterisks indicate comparisons to WT *PTEN* vector. Scale bar in (G') = 100 µm.

**Table S1: *Nkx2.1-Cre*-lineage counts at P30 for the hippocampus, neocortex, striatum and globus pallidus. Related to Figure 1.**

(Top panel) Quantification of cell density (cells/mm$^2$) in the *Nkx2.1-Cre*-lineage (tdTomato$^+$) comparing wild type and *Pten*$^{Flox/Flox}$ brains. (Second panel) Quantification of cell density (cells/mm$^2$) for tdTomato$^+$ cells that co-express PV, SST or ChAT. (Third panel) Quantification of the % tdTomato+ cells that co-express PV, SST or ChAT. (Bottom panel) Quantification of the tdTomato$^+$/PV$^+$ cells per mm$^2$ over tdTomato$^+$/SST$^+$ cells per mm$^2$ in the regions that contained both cell types.

**Table S2: Comparison of the changes in the proportion of PV and SST among different experimental approaches. Related to Figures 1, 6, 7 and S2.**

Quantification of the different experimental approaches that deleted *Pten* from MGE cells and the resulting proportion of cells that expressed either PV or SST. Strategies that analyzed neocortical tissue from conditional mouse models (1st and 2nd columns) showed increased proportion of cells that express PV and decreased SST. Transplantation of MGE cells also resulted in an increased proportion of PV and decreased SST using either MGE cells from the *DlxI12b-Cre; Pten* conditional knockout mouse (3rd column) or by introducing Cre to the MGE via lentivirus (4th column).

**Table S3: Cell intrinsic properties of *DlxI12b-Cre; Pten* cKO transplanted MGE cells at 45 DPT. Related to Figure 6.**

*DlxI12b-Cre*$^+$*; Ai14*$^{Flox/+}$ E12.5 MGE cells that were either *Pten*$^{+/+}$ or *Pten*$^{Flox/Flox}$ were transplanted into WT P1 host neocortices. The Cre$^+$ interneurons (tdTomato$^+$) were assessed for cell intrinsic

firing properties at 45 days post transplant (DPT) and categorized into non-accommodating and accommodating groups. Data are represented as mean ± SEM, *p* values were determined by student's t-Test and are listed to the right of each parameter. Abbreviations: action potential (AP), frequency/injected current (F/I), hertz (Hz), interspike interval (ISI), megaohm (mOhm), millisecond (ms), millivolt (mV).

**Supplemental References**

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